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Full Paper

Isolation and characterization of *Streptococcus thermophilus* possessing *prtS* gene from raw milk in Japan

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Streptococcus thermophilus **is widely used for producing fermented dairy products such as yogurt and cheese. Some *S. thermophilus* strains possessing the cell-wall protease PrtS show high proteolytic activity and fast acidification properties, which are very useful in industrial starters. However, few *S. thermophilus* strains possessing the *prtS* gene have been isolated from the environment. To clarify whether or not *S. thermophilus* strains possessing the *prtS* gene are present in Japan, we isolated *S. thermophilus* from raw milk collected in Japan from 2011 to 2017 and investigated the strains for the presence of *prtS* by PCR. A total of 172 *S. thermophilus* strains were isolated, and 59 strains were confirmed to possess *prtS*. We measured fermentation times of 59 *prtS*-positive strains in skim milk broth and found that 53 strains showed fast acidification properties, finishing fermentation within 10 hr. However, the remaining 6 *prtS*-positive strains showed slow acidification properties, and they had several amino acid mutations in PrtS compared with fast acidifying *S. thermophilus* LMD-9 and 4F44. These results demonstrate that *S. thermophilus* strains possessing *prtS* are prevalent in Japan and that some *prtS*-positive strains could lose their fast acidifying properties through mutations in PrtS.**

Key words: ***Streptococcus thermophilus*****, *prtS*****, yogurt fermentation, mutation**

INTRODUCTION

Yogurt is traditionally fermented by a symbiotic starter culture of *Lactobacillus delbrueckii* subsp. *bulgaricus* (*L. bulgaricus*) and *Streptococcus thermophilus* [1]. Both bacteria provide metabolites which promote mutual growth, as the nutrients in milk are not sufficient for their optimal growth [2, 3]. For example, *L. bulgaricus* produce peptides by degrading milk protein using the cell-wall protease PrtB, supporting the growth of weakly proteolytic *S. thermophilus* [4, 5]. However, a minority of *S. thermophilus* strains have been reported to show high proteolytic activity and fast acidification properties [6, 7]. Such *S. thermophilus* strains possess the *prtS* gene coding the cell-wall protease PrtS, which hydrolyzes casein into peptides [8]. Since *S. thermophilus* possessing *prtS* (*prtS*+) can acidify milk fast without the support of *L. bulgaricus*, *prtS*+ strains have a wide range of applications as industrial starters.

Despite its growth advantage in milk, only a small number of *prtS*+ strains have been reported so far. For example, only 21 among 135 *S. thermophilus* strains of the INRA historical collection were found to be *prtS*+ [9]. The first *prtS*+ strain was isolated from yogurt in Japan in 1971, and the second was isolated from fermented food in Mongolia in 1974 [7], suggesting the possibility that *prtS*+ strains have been prevalent in East Asia. However, to the best of our knowledge, no studies on the isolation of *prtS*+ strains from the environment have been carried out in these areas.

The aim of the present study was to clarify the prevalence of *prtS*+ strains in Japan, an island country in East Asia, by isolating *S. thermophilus* from raw milk collected from its 8 regions. Furthermore, we also measured the fermentation time and investigated the amino acid sequence of *prtS* in the isolated *prtS*+ strains to evaluate the effect of *prtS* on acidification properties.

MATERIALS AND METHODS

Isolation of Streptococcus thermophilus

Raw milk samples were collected from 8 regions of Japan (Fig. 1) from 2011 to 2017, mainly from factories of Meiji Co., Ltd. (Tokyo, Japan). Collected samples were incubated at 48°C for 24 hr under anaerobic conditions. Each sample was diluted with sterile 0.85% (w/w) NaCl solution, and 0.1 mL aliquots were spread on milk agar plates containing 7% (w/w) skimmed milk powder (Meiji Co., Ltd., Tokyo, Japan), 1.9% (w/w) β-glycerophosphate disodium salt (Cayman Chemical, Ann Arbor, MI, USA), and 1.5% Bacto Agar (Becton Dickinson and Company, Franklin

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Lakes, NJ, USA). β-glycerophosphate disodium salt was added to prevent the growth of *Lactobacillus* species [10]. The plates were incubated anaerobically at 48°C for 24 hr. Obtained colonies were isolated by streaking on the abovementioned milk agar plates and incubated anaerobically at 48°C for 24 hr. Colonies were gram stained, and cocci-shaped gram-positive colonies were further incubated on BL agar plates (Nissui Pharmaceutical, Tokyo, Japan) anaerobically at 48°C for 24 hr. DNA was extracted from each colony using InstaGene Matrix (Bio-Rad, Hercules, CA, USA) and identified as *S. thermophilus* by a BLAST search of 16S rDNA and phosphoserine phosphatase gene (*serB*) sequences [11]. Then, RAPD (Random Amplification of Polymorphic DNA) PCR was carried out with strains isolated from the same raw milk sample using the primers RAPD1 (5′-CGTAAATTGC-3′) and RAPD2 (5′-CGTACATTGC-3′) to remove genetically identical strains. The primers used in this study were purchased from Eurofins Japan (Tokyo, Japan). The PCR reaction mixture was prepared as follows: 1 µL genomic DNA from each isolated *S. thermophilus* strain was mixed with 0.4 µL of TaKaRa Ex Taq (Takara Bio, Kusatsu, Shiga, Japan), 2.5 µL of 10×Ex Taq buffer, 2 µL of 2.5 mM dNTP, 0.4 µL of 100 µM primer, and 18.7 µL distilled water. The PCR reaction was performed using a Veriti Thermal Cycler (Thermo Fisher Scientific, Waltham, MA, USA) as follows: 4 cycles of 5 min at 94°C, 5 min at 36°C, and 5 min at 72°C followed by 30 cycles of 1 min at 94°C, 1 min at 36°C, and 2 min at 72°C. Amplified products were then resolved by electrophoresis using 1% agarose L03 gel (Takara Bio, Kusatsu, Shiga, Japan) containing 0.5 µg/mL ethidium bromide (Nippon Gene, Tokyo, Japan) and visualized under UV light by ChemiDoc (Bio-Rad, Hercules, CA, USA). Isolates with the same band pattern were regarded as the same strain, and only isolates showing different band patterns were selected for further investigation.

Fig. 1. Raw milk sampling areas in Japan. The 8 regions of Japan are differentiated by black lines, and each black dot (•) represents a sampling location.

Analysis of prtS

Primers used to check for possession of *prtS* and to sequence the entire coding region of *prtS* are listed in Table 1. The primer set of prtS-1F and prtS-1R was used to amplify 684 bp inside *prtS*. The primer set of prtS-2F and prtS-2R was used to amplify the entire coding region of *prtS*, and the primers prtS-3, 4, 5, 6, 7, 8, and 9 were used for sequencing. The PCR reaction mixture was prepared as follows: 0.5 µL genomic DNA from each isolated *S. thermophilus* strain was mixed with 0.1 µL of Phusion High-Fidelity DNA Polymerase (New England Biolabs, Ipswich, MA, USA), 2 µL of 5×HF buffer, 0.8 µL of 2.5 mM dNTP, 1 µL of 5 µM each primer, and 4.6 µL distilled water. The PCR reaction was performed using a Veriti Thermal Cycler (Thermo Fisher Scientific, Waltham, MA, USA) as follows: 30 sec at 98°C, followed by 30 cycles of 5 sec at 98°C, 20 sec at 63°C, and 3 min at 72°C, and then finally 5 min at 72°C. PCR products were resolved by electrophoresis, and strains showing an amplification product of 684 bp by using the prtS-1F and prtS-1R primers were judged to be *S. thermophilus* possessing *prtS*. For sequencing the entire coding region of *prtS*, PCR products were purified using ExoSAP-IT Express PCR Product Cleanup Reagent (Thermo Fisher Scientific, Waltham, MA, USA) and cycle sequenced by BigDye Terminator v1.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Waltham, MA, USA) using the prtS-3 to 9 primers. Cycle-sequenced products were sequenced using an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster, CA, USA) according to the manufacturer's instructions. Amino acid sequences were predicted and aligned using the GENETYX ver.14 software (Genetyx, Tokyo, Japan) to identify mutations.

Measurement of acidification properties of the isolated strains

Each strain tested was anaerobically precultured twice at 37°C for 18 hr in skimmed milk powder broth containing 10% skimmed milk powder (Meiji Co., Ltd., Tokyo, Japan) and 0.1% yeast extract (Asahi Food and Healthcare, Tokyo, Japan) which was autoclaved at 121°C for 7 min. The preculture was inoculated at 2% (w/w) in 10% skimmed milk powder broth and incubated at 43°C. When fermented under peptide-rich conditions, 0.2% Hyvital Casein CMA 500 (FrieslandCampina, Amersfoort, The Netherlands) was added to 10% skimmed milk powder broth.

Table 1. Oligonucleotide primers used in this study

Primer name	Sequene (5′-3′)
prtS-1F	GACTTGAAGAAACAGCGCGT
prtS-1R	TAGGTGGAGGCGTTACAGTG
prtS-2F	AAGGACGGAGCCATCATGAA
prtS-2R	CGTCCAGTGATGACTTTCCTC
prtS-3	AGAAGAGGAGAAGCTTTCCGT
prtS-4	ATGTGACTGCAGCAATCGAG
prtS-5	TGGCGAGCTAAAACCAGACT
prtS-6	TAGCCGTTCTAGAAGAGCCG
prtS-7	GCAAGCGGTGTGACAACTAT
prtS-8	GACTTGAAGAAACAGCGCGT
prtS-9	ATGACCAGGCAGTTGAAGCA

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Table 2. Number of *S. thermophilus* strains isolated from raw milk

Sampling year	Numbef of raw milk samples	Number of isolated strains								Total number of isolated strains
		Hokkaido	Tohoku	Kanto	Chubu	Kinki	Chugoku	Shikoku	Kyushu	
2011	92	16								16
2012	88	4	6	12	3	1	1	3	7	37
2014	89	71					1	3	5	80
2016	36	7	5	7	6				3	28
2017	8	11								11
Total	313	109	11	19	9	1	2	6	15	172

During fermentation, pH was measured every 5 min using a multiple electrode measuring device (Horiba, Kyoto, Japan) with pH sensor SE 555 (Knick, Berlin, Germany). The time needed for the pH to decline to 4.7 was defined as the fermentation time.

RESULTS

Isolation of S. thermophilus *from raw milk in Japan*

From 2011 to 2017, we collected 313 raw milk samples from all regions of Japan, and a total of 172 colonies were identified as *S. thermophilus*. The number of isolated strains and their isolated regions in each sampling year are summarized in Table 2. We obtained at least 1 strain of *S. thermophilus* from each region in Japan and, of note, over 100 strains of *S. thermophilus* from the Hokkaido region.

Detection of the S. thermophilus *strains possessing* prtS

To examine whether the 172 isolated *S. thermophilus* strains possessed *prtS*, extracted DNA from each strain was amplified by PCR using the prtS-1F and prtS-1R primers. Among the 172 strains, 59 strains showed one amplification product of 684 bp, and the remaining 113 strains showed no products, indicating that 59 strains were *prtS*+ and that 113 strains did not possess *prtS* (*prtS*-). The numbers and percentages of *prtS*+ strains isolated from each region of Japan are summarized in Table 3. The total percentage of *prtS*+ strains among the 172 isolated *S. thermophilus* strains was 34.3%.

Table 3. Number and percentage of *prtS*+ strains

Region	Number of isolated strains	Number of *prtS*+ strains	Percentage of *prtS*+
Hokkaido	109	31	28.4
Tohoku	11	6	54.5
Kanto	19	7	36.8
Chubu	9	5	55.6
Kinki	1	0	0.0
Chugoku	2	1	50.0
Shikoku	6	0	0.0
Kyushu	15	9	60.0
Total	172	59	34.3

Acidification properties of prtS+ *strains*

To examine the acidification properties of the 59 *prtS*+ strains, the fermentation time at 43°C in skimmed milk broth was measured. The number of *prtS*+ strains showing each fermentation time is shown in Fig. 2. Fifty-three *prtS*+ strains showed fast acidification properties, reaching pH4.7 within 10 hr. However, 6 *prtS*+ strains (ME-722, ME-731, ME-751, ME-761, ME-764, and ME-766) needed over 10 hr to reach pH4.7. We also measured the fermentation times of 11 *prtS*- strains isolated in this study and confirmed that all strains needed over 10 hr to reach pH4.7 (data not shown). These results demonstrated that the majority of *prtS*+ strains in Japan were able to acidify milk fast

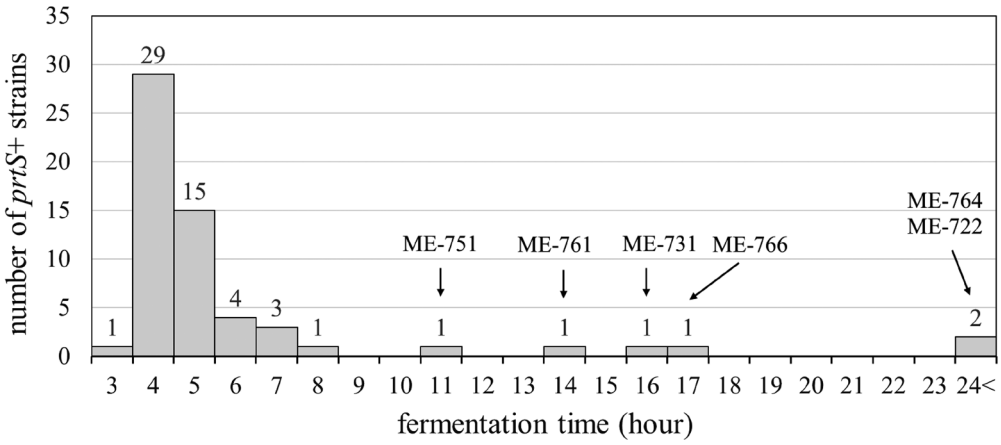

Fig. 2. Number of *prtS*+ strains showing each fermentation time. The names of the 6 strains which needed over 10 hr to reach pH4.7 are described above the bars.

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Table 4. Mutation of PrtS amino acid sequences in slow acidifying *prtS*+ strains

Slow acidifying *prtS*+ strains	Accession No. of *prtS*	Domain	Mutation type	Predicted amino acid change
ME-722	LC514594	H	misssense	Thr1140Ile, Leu1189Ser, Asp1191Glu, Ile1211Val, Ala1232Thr, Glu1294Lys, Ala1342Thr, Tyr1380phe, Asn1424Gly, Val1475Ala
		W	misssense	Ile1533Lys, Glu1548Gly
		AN	misssense	Lys1593Arg, Pro1598Leu, Ile1611Lys
ME-731	LC514595	PP	misssense	Leu38Val, Ala53Val, Gly59Ala
ME-751	LC514596	PP	misssense	Leu38Val, Ala53Val, Gly59Ala
		PR	frameshift	Ala316Serfs*13
ME-761	LC514597	PP	frameshift	Leu26Phefs*12
ME-764	LC514598	SS	misssense	Val19Ile
		A	frameshift	Thr650Asnfs*41
ME-766	LC514599	A	misssense	Asn937Asp, Asp944Asn
		A	nonsense	Trp958*

Predicted PrtS amino acid sequences of 6 slow acidifying *prtS*+ strains (accession Nos. are listed in the table) were compared with those of the PrtS of *S. thermophilus* LMD-9 (accession No. ABJ66087) and 4F44 (accession No. ADB77872). Domain names are abbreviated as follows: PP: signal sequence; PR: catalytic domain; A: globular domain; H: helical domain; W: wall domain; AN: anchored domain [8].

but that a few *prtS*+ strains showed slow acidification properties like *prtS*- strains.

Sequencing of the prtS *of slow acidifying* prtS+ *strains*

To investigate the factors affecting the slow acidification properties of the 6 *prtS*+ strains, amino acid sequences of PrtS were predicted using sequence data of *prtS* and compared with those of two fast acidifying *prtS*+ strains, *S. thermophilus* LMD-9 and *S. thermophilus* 4F44 [12]. Amino acid sequences of the 6 *prtS*+ strains that differed from those of both LMD-9 and 4F44 are listed in Table 4. The presence of a stop codon was confirmed for 4 strains, 3 of which (ME-751, ME-761, and ME-764) had a frameshift mutation and 1 of which (ME-766) had a nonsense mutation. The remaining 2 strains (ME-722 and ME-731) had several missense mutations. These results indicated that slow acidifying *prtS*+ strains had several mutations in PrtS compared with fast acidifying *prtS*+ strains, which might result in the loss of protease activity of PrtS.

Fermentation properties of slow acidifying prtS+ *strains under milk peptide-rich conditions*

To verify the hypothesis that the amino acid mutations in PrtS of slow acidifying *prtS*+ strains resulted in the loss of protease activity and the lower availability of peptides, the fermentation times of the 6 slow acidifying *prtS*+ strains were measured with the addition of casein peptide. As shown in Table 5, the fermentation times of all 6 strains were shortened by adding casein peptide to the medium. Of note, 4 strains (ME-751, ME-761, ME-764, and ME-766) finished fermentation within 10 hr, showing acidification properties like fast acidifying *prtS*+ strains. However, two strains (ME-722 and ME-731) needed over 10 hr even when casein peptide was added to the medium.

Table 5. Fermentation times of *prtS*+ strains with or without casein peptide

Slow acidifying *prtS*+ strains	Fermentation time to pH4.7	
	No addition	Addition of casein peptide
ME-722	>24 hr	15 hr 50 min
ME-731	16 hr 45 min	12 hr 20 min
ME-751	11 hr 55 min	3 hr 45 min
ME-761	14 hr 05 min	3 hr 45 min
ME-764	>24 hr	5 hr 15 min
ME-766	17 hr 05 min	9 hr 45 min

DISCUSSION

The main purpose of this study was to isolate and clarify the prevalence of *prtS*+ strains in Japan and to characterize the acidification properties of each *prtS*+ strain. We isolated 172 *S. thermophilus* strains from 313 raw milk samples in Japan and revealed that 59 strains were *prtS*+. Among the 59 *prtS*+ strains, 53 showed fast acidification properties, whereas the remaining 6 strains needed over 10 hr to finish fermentation. These results demonstrated that *prtS*+ strains could be isolated from raw milk in Japan and that most of them had the ability to ferment milk fast without the support of other proteolytic bacteria.

The percentage of *prtS*+ strains isolated from 2011 to 2017 in this study was 34.3% (Table 3). This value is higher than that in the INRA historical collection collected from 1956 to 2008 (15.6%) [9]. This is also higher than that in the report of Urshev *et al.*, who demonstrated that only 4 strains were *prtS*+ among 20 samples of homemade yogurt, 8 industrial starters, and 80 culture strains collected from 1970 to 1997 [13]. Nowadays, *prtS*+ strains are widely used as commercial starters for fermented foods such as yogurt and cheese due to their fast acidification properties. To obtain fast acidifying *prtS*+ strains, Dandoy *et al.* proposed not only selection but also a natural transformation method in which a 15 kb *prtS* genomic island is transferred to a *prtS*− strain [14]. The higher percentage of *prtS*+ strains in this study than in the previous reports could be attributed to the sampling years (2011–2017), as selection and creation of *prtS*+ strains for industrial starters would have been carried out after the first characterization of *prtS*+ strains in 1991 [6]. It is possible that selected or created *prtS*+ strains somehow made their way into the natural environment and have been increasing due to their

fast-growth properties. Moreover, it could be suggested that some *prtS*− strains acquired the *prtS* genomic island from *prtS*+ strains through naturally occurring horizontal gene transfer, since the *prtS* genomic island is flanked by two tandem sequences of IS elements [9]. Continuous isolation of *S. thermophilus* from the environment and examination for possession of the *prtS* gene would give us important information about the spread of *prtS*+ strains.

Although 53 *prtS*+ strains isolated in this study finished fermentation within 10 hr, the fermentation times varied among the strains (Fig. 2). Galia *et al.* reported that two strains with the same allele of *prtS* showed different acidification rates in milk [12] and confirmed that the expression of *prtS* was higher in the fast acidifying strain than in the slow acidifying strain [15]. They also reported that the expression levels of other genes known to be involved in carbon and nitrogen metabolism were also high in the fast acidifying strain. It can thus be suggested that the variety of acidification properties among the 53 *prtS*+ strains originated from not only the sequence variety of *prtS* but also differences at the transcriptional level of *prtS* and/or other genes involved in carbon and nitrogen metabolism. Though we acquired *prtS* sequence data of slow acidifying *prtS*+ strains, analysis of fast acidifying *prtS*+ strains will be needed to comprehend the differences in acidification properties among strains.

Among the 59 *prtS*+ strains, 6 strains showed slow acidification properties and needed over 10 hr to finish fermentation (Fig. 2), and they had several amino acid mutations in PrtS (Table 4). The acidification properties were recovered by the addition of casein peptides in the strains with a nonsense mutation (ME-751, ME-761, ME-764, and ME-766). Fernandez-Espla *et al.* demonstrated that PR (catalytic domain), followed by A (globular domain), was the best conserved domain between PrtS and other cell-wall proteases of lactic acid bacteria [8]. All 4 strains with a nonsense mutation had a stop codon in PR or A, indicating that defects in these domains led to the dysfunction of PrtS. However, peptide addition was not so effective for 2 strains with missense mutations (ME-722 and ME-731; Table 5). That is, strains with a nonsense mutation in PrtS resulted in lowered protease activity, but strains with missense mutations in PrtS probably had another factor affecting the fermentation properties. One possible factor affecting the fermentation properties of ME-722 and ME-731 might be functional defects in the peptide transporting process or peptide metabolizing process. Three oligopeptide-binding proteins involved in transporting oligopeptide [16] and 15 different peptidase activities [17] were reported in *S. thermophilus*, and these might affect the usage of an added peptide. Another possible explanation is that the metabolic pathways of other nutrients indispensable for growth are impaired in ME-722 and ME-731. Derzelle *et al.* demonstrated that enzymes involved in the synthesis of purines as well as enzymes involved in the supply of amino acids were upregulated in *S. thermophilus* grown in milk [18], which indicated that purines were also important nutrients for growth. It might be possible that lowered acidification properties of ME-722 and ME-731 were caused by factors other than peptide utility, such as glycolysis or purine metabolism.

Delorme *et al.* hypothesized that *S. thermophilus* acquired the *prtS* genomic island by horizontal gene transfer from other streptococcal strains [9]. It remains unclear how and when *prtS*+ strains in Japan acquired *prtS* and why some strains lost the proteolytic function of PrtS by mutation despite the fact that it would result in weak growth. We conducted a phylogenetic analysis of obtained 172 *S. thermophilus* strains by multilocus sequence analysis using 6 genes (*ddlA*, *glcK*, *proA*, *ptsI*, *serB*, *tkt*) by referring to a previous report [19], but there were no cluster correlations with the sampling area, possession of *prtS*, or sampling year (data not shown). We noticed that not only *prtS*− but also *prtS*+ strains were isolated together with proteolytic lactic acid bacteria such as *Lactococcus lactis* subsp. *lactis* and *Lactobacillus helveticus,* indicating that peptide availability might not be the key factor for acquiring *prtS.* Bassi *et al.* reported another function of PrtS, that is, a function which mediates the adhesion of *S. thermophilus* to surfaces conditioned by milk and the involvement in biofilm formation in the dairy environment [20]. To obtain deep insight into environmental conditions affecting gain or loss of *prtS,* further research will be necessary in the future.

In conclusion, we demonstrated that fast acidifying *S. thermophilus* possessing *prtS* were prevalent in Japan. To the best of our knowledge, this is the first report on the isolation of *prtS*+ strains from the environment in Japan. Since fast acidifying *S. thermophilus* strains can be used as important industrial dairy starters, future work will be needed to examine detailed fermentation properties such as urease activity, production of EPS, and organic acids involved in flavor.

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REFERENCES

1. Moon NJ, Reinbold GW. 1976. Commensalism and competition in mixed cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. J Milk Food Technol 39: 337–341. [CrossRef]
2. Zourari A, Accolasa J, Desmazeaud MJ. 1992. Metabolism and biochemical characterisitics of yogurt bacteria. Lait 72: 1–34. [CrossRef]
3. Sieuwerts S, de Bok FA, Hugenholtz J, van Hylckama Vlieg JE. 2008. Unraveling microbial interactions in food fermentations: from classical to genomics approaches. Appl Environ Microbiol 74: 4997–5007. [Medline] [CrossRef]
4. Rajagopal S, Sandine W. 1990. Associative growth and proteolysis of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in skim milk. J Dairy Sci 73: 894–899. [CrossRef]
5. Gilbert C, Atlan D, Blanc B, Portailer R, Germond JE, Lapierre L, Mollet B. 1996. A new cell surface proteinase: sequencing and analysis of the *prtB* gene from *Lactobacillus delbruekii* subsp. *bulgaricus*. J Bacteriol 178: 3059–3065. [Medline] [CrossRef]
6. Shahbal S, Hemme D, Desmazeaud M. 1991. High cell wall-associated proteinase activitiy of some *Streptococcus thermophilus* strains (H-strains) correlated with a high acidification rate in milk. Lait 71: 351–357. [CrossRef]
7. Shahbal S, Hemme D, Renault P. 1993. Characterization of a cell envelope-associated proteinase activity from *Streptococcus thermophilus* H-strains. Appl Environ Microbiol 59: 177–182. [Medline] [CrossRef]
8. Fernandez-Espla MD, Garault P, Monnet V, Rul F. 2000. *Streptococcus thermophilus* cell wall-anchored proteinase: release, purification, and biochemical and genetic characterization. Appl Environ Microbiol 66: 4772–4778. [Medline] [CrossRef]
9. Delorme C, Bartholini C, Bolotine A, Ehrlich SD, Renault P. 2010. Emergence of a cell wall protease in the *Streptococcus thermophilus* population. Appl Environ Microbiol 76: 451–460. [Medline] [CrossRef]
10. Shankar PA, Davies FL. 1977. Recent developments in yogurt starters: a note on the supression of *Lactobacillus bulgaricus* in media containing β-glycerophosphate and application of such media to selective isolation of *Streptococcus thermophilus* from yogurt. Int J Dairy Technol 30: 28–30. [CrossRef]
11. El-Sharoud WM, Delorme C, Darwish MS, Renault P. 2012. Genotyping of *Streptococcus thermophilus* strains isolated from traditional Egyptian dairy products by sequence analysis of the phosphoserine phosphatase (*serB*) gene with phenotypic characterizations of the strains. J Appl Microbiol 112: 329–337. [Medline] [CrossRef]

12. Galia W, Perrin C, Genay M, Dary A. 2009. Variability and molecular typing of *Streptococcus thermophilus* strains displaying different proteolytic and acidifying properties. Int Dairy J 19: 89–95. [CrossRef]
13. Urshev Z, Ninova-Nikolova N, Ishlimova D, Pashova-Baltova K, Michaylova M, Savova T. 2014. Selection and characterization of naturally occurring high acidification rate *Streptococcus thermophilus* strains. Biotechnol Biotechnol Equip 28: 899–903. [Medline] [CrossRef]
14. Dandoy D, Fremaux C, de Frahan MH, Horvath P, Boyaval P, Hols P, Fontaine L. 2011. The fast milk acidifying phenotype of *Streptococcus thermophilus* can be acquired by natural transformation of the genomic island encoding the cell-envelope proteinase PrtS. Microb Cell Fact 10 Suppl 1: S21. [Medline] [CrossRef]
15. Galia W, Jameh N, Perrin C, Genay M, Dary-Mourot A. 2016. Aquisition of PrtS in *Streptococcus thermophilus* is not enough in certain strains to achieve rapid milk acidification. Dairy Sci Technol 96: 623–636. [CrossRef]
16. Garault P, Le Bars D, Besset C, Monnet V. 2002. Three oligopeptide-binding proteins are involved in the oligopeptide transport of *Streptococcus thermophilus*. J Biol Chem 277: 32–39. [Medline] [CrossRef]
17. Rul F, Monnet V. 1997. Presence of additional peptidases in *Streptococcus thermophilus* CNRZ 302 compared to *Lactococcus lactis*. J Appl Microbiol 82: 695–704. [Medline] [CrossRef]
18. Derzelle S, Bolotin A, Mistou MY, Rul F. 2005. Proteome analysis of *Streptococcus thermophilus* grown in milk reveals pyruvate formate-lyase as the major upregulated protein. Appl Environ Microbiol 71: 8597–8605. [Medline] [CrossRef]
19. Delorme C, Legravet N, Jamet E, Hoarau C, Alexandre B, El-Sharoud WM, Darwish MS, Renault P. 2017. Study of *Streptococcus thermophilus* population on a world-wide and historical collection by a new MLST scheme. Int J Food Microbiol 242: 70–81. [Medline] [CrossRef]
20. Bassi D, Cappa F, Gazzola S, Orrù L, Cocconcelli PS. 2017. Biofilm formation on stainless steel by *Streptococcus thermophilus* UC8547 in milk environments is mediated by the proteinase PrtS. Appl Environ Microbiol 83: e02840–16. [Medline] [CrossRef]

